ELSEVIER

Cancer Genetics and Cytogenetics 130 (2001) 75–78

CANCER GENETICS AND CYTOGENETICS

Short communication

FISH characterization of t(8;12)(q12;p13) observed as the sole karyotypic anomaly in a myelodysplastic syndrome patient

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Received 21 December 2000; accepted 16 February 2001

Abstract We report a t(8;12)(q12; p13) as the sole cytogenetic anomaly in a patient with a myelodysplastic syndrome (MDS). By means of FISH, we mapped the genomic region involved in the breakpoint (bkp) on both chromosomes. The 12p13 bkp mapped between markers WI-664 and WI-9218, immediately distal to the breakpoint cluster region frequently involved in hematological neoplasms targeted by y964C10. The 8q12 bkp (not yet investigated by FISH) was characterized and found to occur between markers WI-3263 and D8S524 within the region recognized by y874E10.

1. Introduction

Numerical and structural rearrangements of chromosomes 8 and 12 have been described in hematological malignancies as different as acute lymphoblastic (ALL), acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) or myeloproliferative disorders, and non-Hodgkin lymphomas [1]. The breakpoints of translocations and dicentrics involving 12p are most frequently localized in 12p13 within the *ETV6* gene (also known as *TEL*), a member of the ETS family of transcription factors, whose involvement in leukemia is particularly interesting insofar as different translocations are associated with distinct forms of disease [2]. To mention just a few of the partner bands and genes that are affected in different hematological conditions, *ETV6* is fused to the platelet-derived growth factor β receptor in a case of chronic myelomonocytic leukemia carrying a t(5;12) [3] and to the runt-related gene *CBFA2/AML1*, which is affected by the most common t(12;21) in pediatric acute lymphoblastic leukemia [4]. The loss of 12p material is often associated with an ETV6 translocation and is sometimes accompanied by the concomitant deletion of the other ETV6 allele [5], a feature linking this rearrangement to a 12p interstitial deletion [6,7]. As far as chromosome 8 is concerned, trisomy 8 is the most frequent numerical aberration in myeloid disorders, including acute myelogenous leukemia (AML), myeloproliferative disorders, and MDS [1,8]. Chromosome translocations are rare in MDS, with the exception of t(8;21)(q12;q22), which is usually found in rapidly evolving MDS [9]. In particular, t(8;12)(q12;p13) has been described in the context of a complex karyotype in a few cases of AML, MDS [6,7,10], and chronic myelogenous leukemia (CML) [6]. We here report the fluorescence in situ hybridization (FISH) characterization of an apparently balanced (8;12)(q12;p13) translocation, identified as the sole cytogenetic lesion in the bone marrow karyotype of a patient with MDS (FAB subtype: refractory anemia with an excess of blasts, RAEB). Using YAC probes located to 8q12 and 12p13, we precisely defined the genomic region involved in the breakpoints on both chromosomes.

2. Case report

A 65-year-old female was admitted to our institution in 1994 for pancytopenia. The laboratory data showed anemia (8 g/dl), thrombocytopenia (60×10^9/l), and leukopenia (2.8×10^9/l, with neutrophils 1.1×10^9/l). A bone marrow aspirate and biopsy detected 9% of myeloid blasts. A diagnosis of RAEB was made on the basis of the FAB Cooperative Group diagnostic criteria. The patient presented with splenomegaly (ultra-

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0165-4608/01/$ – see front matter
PII: S0165-4608(01)00456-3

sound bipolar axis of 20 cm), and was dependent on packed red blood cell transfusions. One course of polychemotherapy with daunorubicin, cytosine arabinoside, and 6-thioguanine induced a complete remission after prolonged bone marrow aplasia complicated by life-threatening pneumonia. In 1995, a splenectomy was performed in an attempt to reduce red blood cell transfusions, but without a lasting response. Between 1995 and 1999, the patient was affected by numerous viral (*herpes zoster)*, mycotic *(aspergillus*), and bacterial infections (*pseudomonas*); she died of fungal sepsis in 1999.

3. Materials and methods

3.1. Cytogenetics and FISH analysis

Chromosomes were prepared from bone marrow cultures. The cytogenetic analysis was performed using the QFQ banding technique, following the International System for Human Cytogenetic Nomenclature [11].

The YAC probes, used in FISH experiments from the CEPH YAC library [12], were provided by the YAC Screening Centre (Dibit, HSR, Milan, Italy). FISH experiments were performed following the protocols of Lichter et al. [13] and Lichter and Cremer [14], with minor modifications.

4. Results

Cytogenetic analysis, performed according to standard methods on 50 metaphases from bone marrow cells, revealed a 46,XX,t(8;12)(q12;p13) acquired karyotype. In order to refine the localization of the 8q12 and 12p13 translocation breakpoints, we performed FISH analyses using yeast artificial chromosome clones (YACs) mapping to these chromosomal bands. Table 1 shows the YAC clones used and the FISH results. The breakpoint on chromosome 8 was investigated by means of 14 overlapping YAC clones belonging to the WC8.7 contig and encompassing the 8q11.2–8q12 bands. The FISH signals of y874E10 were observed on both derivative chromosomes, thus indicating that this YAC encompasses the breakpoint region on the der(8) (Fig. 1a). In order to refine the 8q12 bkp mapping further, we identified two additional YAC clones overlapping the centromeric (y820C4) and telomeric (y849B4) portions of y874E10 (Fig. 1d and Table 1). As y820C4 hybridized only to the der(8) and y849B4 hybridized only to the der(12), we were able to locate the breakpoint between markers WI-3263 and D8S524, which are, respectively, reported to map to the telomeric end of y820C4 and the centromeric end of y849B4 (Table 1, Fig. 1d, and data not shown). To map the chromosome 12 breakpoint, we used six YAC clones anchored to the contig WC12.1 and mapping to 12p13 (Table 1). As FISH signals of y746A12 were detected on the der(8) (Figs. 1b and 1e) and those of y964C10 were detected on the der(12) (Figs. 1c and 1e), the 12p13 breakpoint appears to be delimited by marker WI-9218 mapping to the centromeric end of y746A12 and marker WI-664 mapping to the telomeric end of y964C10 (Fig. 1e). These results were confirmed by hybridization with the contiguous y817H1, which almost completely overlaps y964C10 [15] (Fig. 1e).

Table 1
YAC FISH results on derivative chromosomes

YAC name	Localization	FISH signal on derivative chromosomes
935D11	8q11.2	der(8)
787A4	8q11.2	der(8)
791E12	8q11.2	der(8)
820C4[a]	8q11.2	der(8)
874E10[a]	8q12	der(8)/der(12)
849B4[a]	8q12	der(12)
834A9	8q12	der(12)
935E9	8q12	der(12)
946B7	8q12	der(12)
925G7	8q12	der(12)
817B1	8q12	der(12)
799C11	8q12	der(12)
787A6	8q12	der(12)
953G3	8q12	der(12)
929E11	12p13	der(12)
964a12[a]	12p13	der(12)
817H1[a]	12p13	der(12)
746A12[a]	12p13	der(8)
808E9	12p13	der(8)
886D1	12p13	der(8)

YAC order on 8q and 12p is given from tel to cen.
[a]YACs shown in Figs. 1d–1e.

5. Discussion

A few cases of AML, MDS [6,7,10] and CML [6] have been described harboring the translocation t(8;12)(q12;p13), usually associated with a complex karyotype. We here report a case of MDS (FAB subtype: refractory anemia with an excess of blasts, RAEB), in which a balanced t(8;12)(q12;p13) was the sole cytogenetic lesion in the acquired bone marrow karyotype. Given the possible pathogenetic relevance of unique reciprocal translocations in human leukemia, the regions involved in the breakage, giving rise to the recombinant der(8) and der(12) chromosomes, were characterized by FISH analysis using YAC probes targeting 8q12 and 12p13. This made it possible to map the breakpoints on both partner chromosomes precisely. We identified a molecular probe y874E10 (encompassing the 8q12 breakpoint), which mapped between markers WI-3263 and D8S524. YAC 874E10 may prove to be a useful reagent for FISH characterization of the 8q12 region, which has not yet been targeted in myelodysplasia and AML. We also pinpointed the 12p13 breakpoint between markers WI-664 and WI-9218, which delimit a genomic region immediately telomeric to that identified by y964C10 as containing the *ETV-6* gene that is most frequently involved in deletions and translocations in leukemia [3,6,16]. Two other breakpoint cluster regions, occurring approximately 4 cM distal to the bkp here described, have been reported, thus indicating that the overall 12p13 cytogenetic band is prone to rearrangement [17]. Although y964C10 did not seem to be affected in the translocation of our case, the

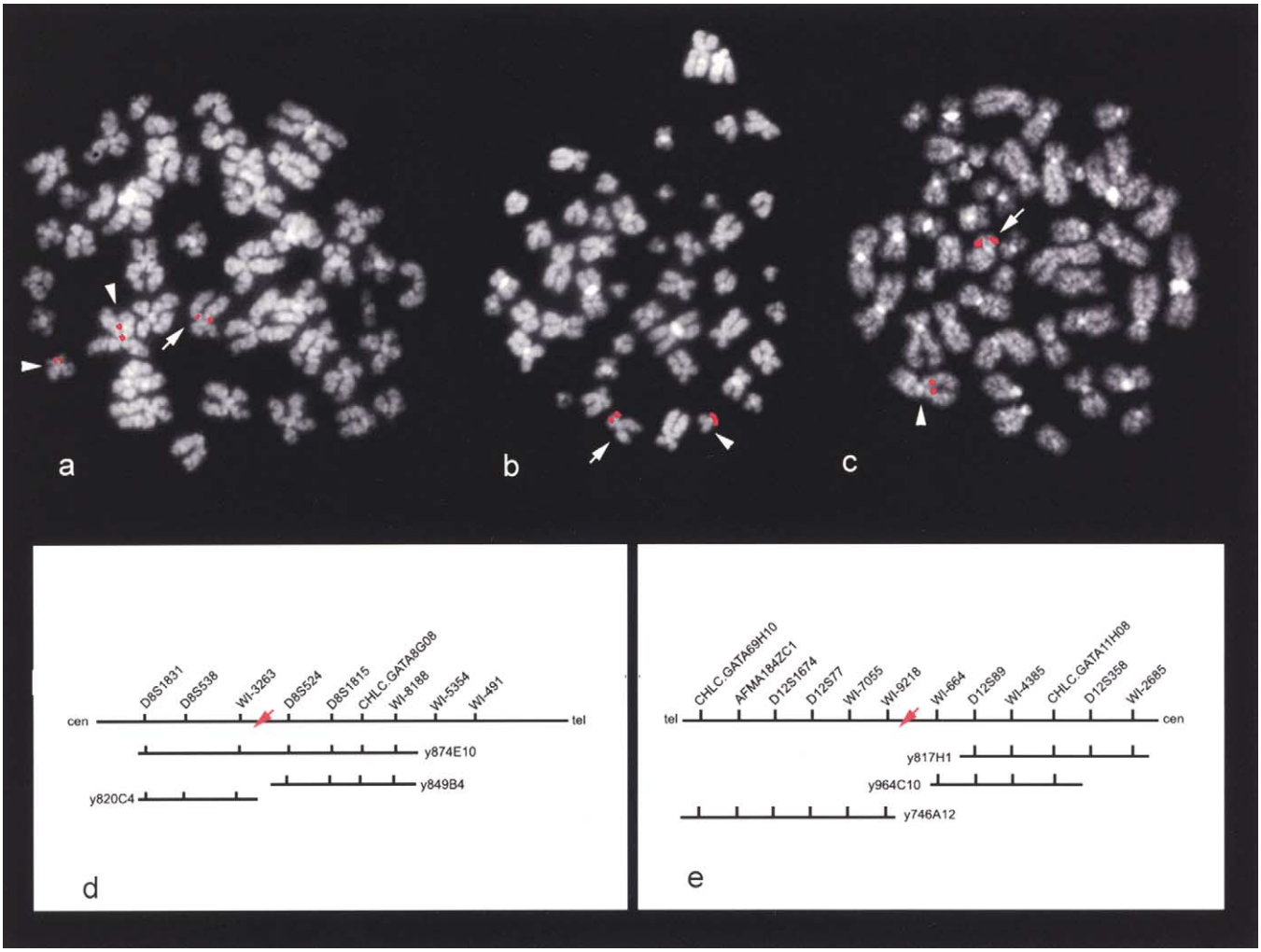

Fig. 1. Characterization by YAC FISH of reciprocal translocation breakpoints leading to der(8) and der(12) chromosomes (a) y874E10 (8q12) shows a split signal on der(8) and der(12) (arrowheads), thus indicating that it spans the bkp on chromosome 8 (normal chromosome 8 identified by arrow); (b) y746A12 mapping to 12p13 shows a "moved" hybridization signal on chromosome der (8) (arrowhead) targeting a region distal to the translocation bkp on 12p13 (normal chromosome 12 indicated by arrow); (c) y964C10, contiguous to y746A12 on 12p13 gives the expected signal on der(12) (arrowhead), thus fixing the centromeric boundary of the translocation bkp on chromosome 12 (arrow). (d) Physical map of the breakpoint regions at 8q12 and (e) at 12p13. The loci are reciprocally ordered as shown in the MIT web sit (http://www.genome.wi.mit.edu), but the distances are not to scale. Red arrows point to the regions involved in the breakpoints.

possible involvement of small ETV-6 deletions that escape detection by YAC FISH cannot be ruled out.

In conclusion, our data confirm the association between MDS and 12p13 genetic lesions, particularly t(8;12)(q12; p13). They also provide the first FISH characterization of the 8q12 breakpoint using an 8q12-specific probe that may prove valuable in estimating the frequency of the involvement of this genomic region in MDS. The availability of patients bearing 8q12 rearrangements may allow the refined mapping of the breakpoint and favor the search for candidate genes affected in myelodysplasia.

Acknowledgments

The authors thank the YAC Screening Centre of Dibit, HSR, Milan, Italy, for providing all of the YAC clones used in this study. The work was partially supported by AIL (Associazione Italiana contro le Leucemie) to GLD and by a grant to Istituto Auxologico Italiano IRCCS from the Ministry of Health.

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